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Characterization of adiponectin concentrations and molecular weight forms in serum, seminal plasma, and ovarian follicular fluid from cattle

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ABSTRACT

Adiponectin (AdipoQ), an adipocyte-derived hormone, is one of the most abundant adipokines in the blood circulation. Adiponectin has various metabolic functions, such as improving insulin sensitivity in humans and rodents. The role of AdipoQ in reproduction is not yet fully understood, but the expression of AdipoQ in reproductive tissues has been observed in various animals and humans, including chicken testis, bovine ovary, and human placenta. The objective of this study was to characterize AdipoQ in the bovine body fluids related to reproduction. Therefore, we evaluated the seminal plasma (SP) from breeding bulls ($n = 29$) and follicular fluid (FF) from heifers ($n = 14$), and we also collected blood samples from these animals. In addition, blood samples from other bulls ($n = 30$) and heifers ($n = 14$) were assayed for AdipoQ. The concentrations were assessed using a bovine-specific ELISA, and the molecular weight (MW) pattern of the AdipoQ protein was estimated by the Western blot analysis. The SP AdipoQ concentrations were approximately 180-fold lower compared with that in the serum concentrations. Furthermore, the AdipoQ concentrations in the serum and SP were positively correlated. The MW patterns of AdipoQ in the serum and SP were different such that the high MW form of AdipoQ was more abundant in the SP than serum. The AdipoQ concentrations in the serum and SP also increased with age: old bulls (>6 years) had higher AdipoQ concentrations in the serum and SP than bulls aged 24 months or lesser ($P < 0.05$). In the FF, the AdipoQ concentrations were 1.6-fold lower than those in the corresponding serum samples, and the concentrations in the serum and FF were not correlated ($P > 0.1$). In the FF, only the middle MW forms of AdipoQ were detectable by Western blotting. The MW pattern in the serum did not differ between the sexes. Our data provide both the AdipoQ concentration and the MW patterns for bovine body fluids related to reproduction.

1. Introduction

Adiponectin (AdipoQ) is one of the most abundant adipokines that is present in the μg/mL range in the blood circulation. Adiponectin is predominantly secreted by adipocytes [1] and has potential benefits on insulin sensitivity

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[2] and also has anti-inflammatory activity [3]. Adiponectin exerts its effects by activating a range of different signaling molecules *via* binding to two transmembrane AdipoQ receptors, AdipoR1 and AdipoR2. AdipoR1 is expressed primarily in the skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver [4]. The major signaling molecules activated by AdipoQ are adenosine monophosphate–activated protein kinase, p38 mitogen-activated protein kinase, and the transcription factors peroxisome proliferator–activated receptor α and nuclear factor kappa-light-chain-enhancer [4]. AdipoR1 acts mainly *via* the APMK pathway, thus inhibiting fatty acid oxidation, whereas AdipoR2 acts through peroxisome proliferator–activated receptor-α signaling, which stimulates fatty acid oxidation [5]. Adiponectin is synthesized as a single 28-kDa monomer but is not secreted as such; instead, it undergoes multimerization to form different molecular weight (MW) multimers before secretion [6]. Adiponectin is found in the circulation in three different MW forms as follows: a trimer with low MW (LMW, 67 kDa), a hexamer with middle MW (MMW, 136 kDa), and a multimeric form of 12 to 18 monomers with high MW (HMW, >300 kDa). The AdipoQ monomer is only detectable after reducing and denaturation [6]. The HMW AdipoQ is considered the most biologically active form [7]. The potential effects of AdipoQ on reproduction have been addressed in various studies. Adiponectin may affect the female and the male reproductive system at different levels, through both central and local regulatory mechanisms. Adiponectin expression was observed in several species in different types of ovarian cells. Adiponectin messenger RNA (mRNA) was detected in chicken theca cells and, to a lesser extent, granulosa cells of follicles during different stages of maturation [8]. In cows, AdipoQ mRNA was detected in the theca, granulosa, and cumulus cells and the oocyte [9]. In rat ovaries, the AdipoQ protein was immunohistochemically localized in theca–interstitial cells, CL, and oocytes, and lesser amounts were detected in granulosa cells [10]. By contrast, AdipoQ mRNA was reported to be absent or low in a human granulosa cell line and mouse and human granulosa and cumulus cells [10,11]. Moreover, *in situ* hybridization localized the AdipoQ mRNA in adipose tissue adherent to the ovary but not in any ovarian cells in mice [11]. However, the expression of the AdipoQ receptors AdipoR1 and AdipoR2 was consistently confirmed in ovarian tissues from chicken, mouse, human, and cattle [8,10,11]. Adiponectin may function in the ovary during steroidogenesis, although basal or FSH-stimulated steroidogenesis in primary rat granulosa cells was not affected by treatment with recombinant AdipoQ, and costimulation with insulin or insulinlike growth factor 1 increased both progesterone and estradiol production [10]. By contrast, AdipoQ decreased insulin- or insulinlike growth factor 1–induced progesterone production in bovine granulosa or theca cells [12,13], and these inhibitory effects were primarily located in theca cells [12]. Adiponectin may also function in female reproduction during maturation of the oocyte; however, there are conflicting results. In cattle, AdipoQ did not modify oocyte maturation *in vitro*, whereas the meiotic maturation of pig oocytes derived from follicles (3–6-mm diameter) was supported by recombinant porcine AdipoQ [14]. These results indicate that species differences may exist with regard to the specific ovarian response to AdipoQ.

For male reproduction, the AdipoQ protein was localized in the peritubular cells and Leydig cells of the chicken testis [15]. The Leydig cells were also positive for AdipoQ in the rat testis [16]. Adiponectin expression is less responsive to gonadotropins than metabolic signals, suggesting a function of AdipoQ as the endocrine integrator linking metabolism and gonadal function [16]. AdipoR1 and AdipoR2 mRNA were observed in several testicular cell types and were higher in adult *versus* prepubertal chickens [15]. Androgens, particularly testosterone, are inversely related to the serum AdipoQ concentrations in humans [17] and mice [18], and treatment with recombinant AdipoQ inhibits testosterone secretion *ex vivo* [16].

Human seminal plasma (SP) is the male body fluid related to reproduction and reportedly contains AdipoQ at concentrations approximately 66-fold lower than serum. In addition, a positive correlation between the AdipoQ concentrations in both matrices was observed [19]. In human follicular fluid (FF), the concentrations of AdipoQ reach approximately 20% to 25% of the corresponding serum samples and are positively correlated with the serum values [20,21].

In addition to the differences in AdipoQ concentrations, the isoforms of AdipoQ differ between the serum and the FF. In the FF, the LMW form of AdipoQ is the most abundant fraction, whereas in the serum, the HMW fraction is predominant [22].

Studies of AdipoQ in cattle have been impeded by the lack of validated species-specific assays. There is one report of the serum AdipoQ concentration in bulls that were determined by an ELISA using an antihuman goat antibody [23]. However, the concentrations reported were approximately 100-fold lower than that expected on the basis of the data published for humans and rodents [1].

The present study investigates the AdipoQ concentrations and MW patterns in bovine serum, FF, and SP. Our objectives were as follows: (1) determine the AdipoQ concentrations and MW patterns in the serum and SP of breeding bulls and (2) characterize the AdipoQ in the serum and FF longitudinally during the estrous cycle in heifers.

2. Materials and methods

2.1. Bulls

2.1.1. Seminal plasma and blood samples

Blood and semen samples were collected at the same day from bulls owned by the bull stud center of the cattle breeding association “Rinder Union West e.G.” in Borken, Germany. Blood samples were collected by jugular venipuncture from 59 bulls (Holstein, n = 56; Limousin, n = 1; Pinzgauer, n = 2; average age, 37 ± 31 months [mean ± standard deviation], median = 24 months, and range = 11 months to 12 years). The samples were allowed to clot for 2 hours before centrifugation (×1000*g*, 15 minutes, 4 °C), and the sera obtained were stored at −20 °C until further analysis.